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A novel radiation-resistant *Deinobacter* sp. isolated from irradiated pork

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A red-pigmented, radiation-resistant, Gram-negative, rod-shaped bacterium isolated from irradiated pork is described. The D_{10} values in buffer solution and on pork mince are 3·45 and 5·05 kGy respectively. The strain has been identified as a *Deinobacter* species.

The sensitivity of micro-organisms to ionizing radiation can vary with genus, species or strain. The relative sensitivity of bacteria to gamma irradiation is compared by calculating D_{10} values where the D_{10} is the dose required to inactivate 90% of the population. Gram-negative bacteria tend to be more sensitive than Gram-positive to irradiation (have lower D_{10} values) and rod-shaped bacteria tend to be more sensitive than cocci (Ingram & Farkas 1977). The range of sensitivity of bacteria commonly found in foods is large, for example, the D_{10} value of a *Pseudomonas fluorescens* strain irradiated in a low fat beef at 5°C was 0·13 kGy compared to 0·58 kGy for *Staphylococcus aureus* irradiated under the same conditions (Maxcy & Tiwari 1973). *Clostridium botulinum* type A spores were found to have D_{10} values ranging from 3·4 to 3·6 kGy in cooked beef irradiated at 25°C (Grecz *et al.* 1971).

In addition to these more common food contaminants non-spore-forming, extremely radiation-resistant bacteria are known to occur naturally in foods. *Deinococcus radiodurans*, formerly *Micrococcus radiodurans*, was first isolated by Anderson *et al.* (1956) from meat showing signs of proteolytic spoilage despite exposure to a putative sterilizing dose of gamma irradiation. Similar resistant strains of *D. radiodurans* have since been isolated from air (Murray & Robinow 1958) and fish (Davis *et al.* 1963). The D_{10} values of these bacteria were 2·5–3·08 kGy, when irradiated in raw beef at 5°C (Duggan *et al.* 1963). All the strains were aerobic Gram-positive cocci, oxidase and catalase positive. On routine laboratory culture media the colonies ranged from pink to bright red. Although these organisms stained Gram-positive, the fatty acid profile, and cell wall and outer membrane structure, is similar to Gram-negative species (Brooks *et al.* 1980).

A Gram-negative, red or pink, rod-shaped bacterium has recently been isolated from animal faeces and freshwater fish (Oyaizu *et al.* 1987). The organism, designated as *Deinobacter grandis* gen., sp. nov, has a D_{10} value of 3·6 kGy when irradiated in phosphate buffer. It had a similar cell wall structure and fatty acid profile to the genus *Deinococcus*.

This report describes a similar radiation-resistant, Gram-negative rod isolated from irradiated pork.

Materials and Methods

ISOLATION AND MAINTENANCE OF THE ORGANISM

The organism was isolated on glucose nutrient agar (Oxoid CM3 + 0·5% D-glucose), incubated at 25°C for 3 d, from a pork chop which had

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been irradiated to a dose of 1·75 kGy. Better growth was observed on Yeast extract peptone (YP) agar (Oyaizu & Komagata 1981) and subsequently the organism, which produces a bright red pigment, was cultured on YP broth or agar at 25°C, unless otherwise stated.

DETERMINATION OF RADIATION RESISTANCE

The organism was grown in 500 ml YP broth for 24 h at 25°C, harvested by centrifugation and resuspended in phosphate buffered saline (PBS, Oxoid BR14a) to give approximately 10^8 cells/ml. Aliquots (1 ml) of inoculum were dispensed into sterile stoppered plastic tubes (Sterilin, Middlesex, England) and irradiated using a ^{60}Co Gammabeam 650 facility (Atomic Energy of Canada Limited) with a dose rate of 0·85 kGy/h and a temperature of 15°C. Samples were irradiated in triplicate at doses of 0, 2, 4, 6, 8 and 10 kGy. The number of survivors in each sample was determined by the Miles & Misra (1938) technique on YP agar at 25°C for 3 d. The logarithm of the bacterial count was plotted against radiation dose to determine death rate and the D_{10} value calculated as the reciprocal of the slope of the best fit line (Ley 1983). A similar procedure was used to determine the D_{10} value in sterile pork. Samples (25 g) of irradiation-sterilized (dose of 25 kGy) minced pork were inoculated with 1 ml of culture and mixed well before irradiation in triplicate.

GROWTH CHARACTERISTICS AND BIOCHEMICAL TESTS

A temperature gradient incubator (Toyo Kagaku Sangyo Co., Ltd, Tokyo) was used to determine growth from 0 to 45°C. The optimum growth temperature was assessed by nephelometer readings. Growth under aerobic, anaerobic and microaerophilic conditions was determined on YP agar after 3 d at 25°C. The organism was examined for Gram reaction, motility, colony morphology, the presence of oxidase and catalase, gelatin hydrolysis, argin-

Table 1. Comparison of pork isolate with *Deinobacter grandis**

Test	Pork isolate	*Deinobacter grandis*
Oxidase	+	−
Catalase	+	+
Gelatin hydrolyis	+	+
Arginine hydrolysis	−	N/A†
Aesculin hydrolysis	−	+
Nitrate reduction	−	+
H_2S production	−	−
Urease	−	−
Acid production from glucose:		
aerobically	−	−
anaerobically	−	−
Gram reaction	−	−
Cell size	0·5–0·6 × 2–3·6 μm	0·6–1·2 × 1·5–4 μm
Colony pigmentation	red	pink or red
Motility in 24 h culture	−	−
Growth at:		
5°C	−	−
37°C	+	+
42°C	+	+
Optimum growth range	25–30°C	N/A
Growth on YP agar:		
aerobically	+	+
microaerophilically	+	N/A
anaerobically	−	−
Mol % G + C	60·9–62·9	68·4–69·4
D_{10} value in PBS	3·45 kGy	1·0–3·7 kGy
D_{10} value in pork	5·05 kGy	N/A

* Results from work by Oyaizu *et al.* (1987).
† N/A, data not available.

ine hydrolysis, nitrate reduction H_2S production and oxidation/fermentation of glucose. All tests were carried out using 24 h cultures in YP broth at 25°C. The mol% G + C was determined by the thermal melting method (Marmur & Doty 1962) using DNA extracted from the organism as described by Marmur (1961). Cells were stained with lead citrate and uranyl acetate (Reynolds 1963) and observed using a JEM 1200EX Temscan electron microscope.

Results and Discussion

The D_{10} values (Table 1) obtained in PBS were similar to those of *Deinobacter grandis* and *Deinococcus* spp. (2·5–3·08 kGy). The higher D_{10} value obtained in pork than in PBS is attributed to the presence of protective agents, such as proteins (Jay 1986). The results compared with the description of *D. grandis* are presented in Table 1. After 3 d aerobic incubation at 25°C,

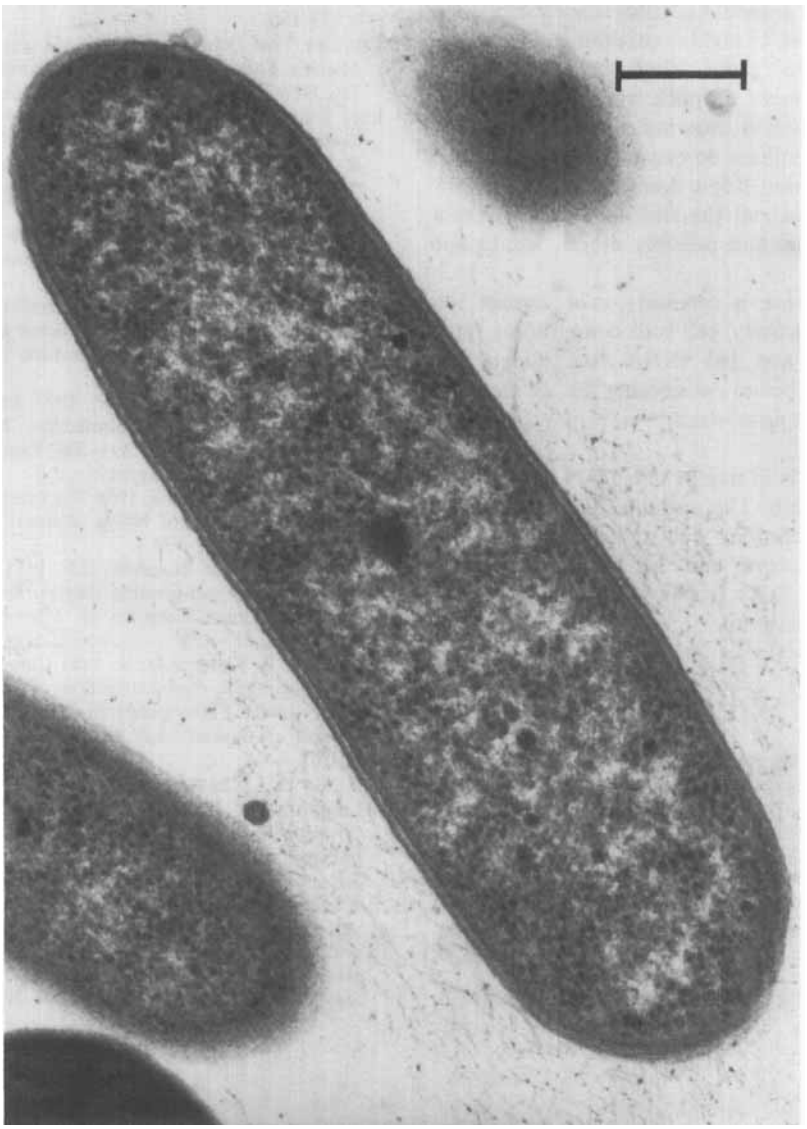

Fig. 1. Transmission electron micrograph of pork isolate showing layered cell wall structure. Bar represents 0·2 μm.

the colonies of YP agar were 2–3 mm in diameter, circular, smooth, convex, entire and opaque with a red pigmentation which was water insoluble. Variation in cell morphology was not observed in older cultures. The sections examined under the electron microscope (Fig. 1) revealed a layered structure to the cell wall, typical of Gram-negative bacteria, but the looped external membranous structure described by Oyaizu *et al.* (1987) was not observed. The results indicate that the strain, although similar, has distinct differences from *Deinobacter grandis* and provides evidence for the occurrence of highly radiation-resistant bacteria of the genus *Deinobacter* on pork. Numbers present on pork were quite low (100 cfu/g), however, it does indicate that radiation-resistant organisms do occur naturally in foods. This means that if low dose irradiation were to be used to extend the shelf-life of fresh meats this organism, and possibly others, would not be killed.

Further work is necessary to determine the proteolytic activity, cell wall composition, fatty acid profile and 16S rRNA catalogue of the pork isolate before considering further the taxonomy of this novel strain.

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References

ANDERSON, A.W., NORDAN, H.C., CAIN, R.F., PARRISH, G. & DUGGAN, D. 1956 Studies on a radio-resistant micrococcus I. The isolation, morphology, cultural characteristics and resistance to gamma radiation. *Food Technology* **10**, 575–577.

BROOKS, B.W., MURRAY, R.G.E., JOHNSON, J.L., STACKEBRANDT, E., WOESE, C.R. & FOX, G.E. 1980 Red-pigmented micrococci: a basis for taxonomy. *International Journal of Systematic Bacteriology* **30**, 627–646.

DAVIS, N.S., SILVERMAN, G.J. & MASUROVSKY, E.B. 1963 Radiation resistant, red-pigmented coccus isolated from haddock tissue. *Journal of Bacteriology*, **86**, 294–298.

DUGGAN, D.E., ANDERSON, A.W. & ELLIKER, P.R. 1963 Inactivation of the radiation-resistant spoilage bacterium *Micrococcus radiodurans* I. Radiation inactivation rates in three meat substrates and in buffer. *Applied Microbiology* **11**, 398–403.

GRECZ, N., WALKER, A.A., ANELLIS, A. & BERKOWITZ, D. 1971 Effect of irradiation temperature in the range −196 to 95C on the resistance of spores of *Clostridium botulinum* 33A in cooked beef. *Canadian Journal of Microbiology* **17**, 135–142.

INGRAM, M. & FARKAS, J. 1977 Microbiology of foods pasteurised by ionising radiation. *Acta Alimentaria* **6**, 123–185.

JAY, J.M. 1986 Food preservation using irradiation. In *Modern Food Microbiology* 3rd edition, pp. 297–316. New York: Van Nostrand Reinhold Company.

LEY, F.J. 1983 Food irradiation. In *Food Microbiology: Advances and Prospects* eds. Roberts, T.A. & Skinner, F.A. The Society of Applied Bacteriology Symposium Series No 11. pp. 113–129. London: Academic Press.

MARMUR, J. 1961 A procedure for the isolation of deoxyribonucleic acid and microorganisms. *Journal of Molecular Biology* **3**, 208–218.

MARMUR, J. & DOTY, P. 1962 Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.

MAXCY, R.B. & TIWARI, N.P. 1973 Irradiation of meats for public health protection. In *Radiation Preservation of Food* pp. 491–504. Vienna: International Atomic Energy Agency.

MILES, A.A. & MISRA, S.S. 1938 The estimation of the bactericidal power of blood. *Journal of Hygiene, Cambridge*, **38**, 732–784.

MURRAY, R.G.E. & ROBINOW, C.F. 1958 Cytological studies of a tetrad-forming coccus. *Proceedings of the International Congress of Microbiology*, 7th, Stockholm, 427–428.

OYAIZU, H. & KOMAGATA, K. 1981 Chemotaxanomic and phenotypic characterization of the strains of species in the Flavobacterium-Cytophaga complex. *Journal of General and Applied Microbiology* **27**, 57–107.

OYAIZU, H., STACKEBRANDT, E., SCHLEIFER, K.H., LUDWIG, W., POHLA, H., ITO, H., HIRATA, A., OYAIZU, Y. & KOMAGATA, A. 1987 A radiation-resistant, rod-shaped bacterium *Deinobacter grandis* gen. nov., sp. nov. with peptidoglycan containing ornithine. *International Journal of Systematic Bacteriology* **37**, 62–67.

REYNOLDS, E.S. 1963 The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.